expression system, the cellular distribution of CFP-tagged $hK_{Ca}3.1a$ and/or YFP-tagged $hK_{Ca}3.1b$ isoforms showed that $hK_{Ca}3.1b$ suppressed the localization of $hK_{Ca}3.1a$ to the plasma membrane. In the *Xenopus* oocyte translation system, co-expression of $hK_{Ca}3.1b$ with $hK_{Ca}3.1a$ suppressed IK_{Ca} channel activity of $hK_{Ca}3.1a$ in a dominant-negative manner. In addition, the present study indicated that up-regulation of $mK_{Ca}3.1b$ in mice thymocytes differentiated $CD4^+CD8^+$ phenotype thymocytes into $CD4^-CD8^-$ ones, and suppressed concanavalin-A-stimulated thymocyte growth by down-regulation of mIL-2 transcripts. Anti-proliferative effects and down-regulation of mIL-2 transcripts were also observed in $mK_{Ca}3.1b$ over-expressing mice thymocytes. These suggest that the N-terminal domain of $K_{Ca}3.1$ is critical for channel trafficking to the plasma membrane, and that the fine tuning of IK_{Ca} channel activity modulated through alternative splicing may be related to the control in physiological and pathophysiological conditions in T-lymphocytes.

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Phosphoinositides Control the Activity of Slick and Slack K^+ Channels

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Phosphoinositides are acidic membrane phospholipids involved in cell signaling in eukaryotes. The most common isoform in the plasma membrane, phosphatidylinositol 4,5-biphosphate, usually named PIP_2, has proven to be necessary for the activity of a number of ion channels and transporters. PIP_2 regulates all members of the Kir and KCNQ channel families; furthermore it also directly modulates Slo1 (BK_{Ca}) and Slo3 channels. Slick (Slo2.1) and Slack (Slo2.2) channels are high-conductance K^+ channels widely distributed in the CNS. The present work evaluates the PIP_2 sensitivity of Slo2 channels, namely Slick and Slack K^+ channels. To address this issue, both channels were expressed in *Xenopus laevis* oocytes and currents were measured by two-electrode voltage clamp before and after pre-incubation of oocytes in 10μM PIP_2. Two isoforms of PIP_2: $PI_{(3,4)}P_2$ and $PI_{(4,5)}P_2$ activated K^+ currents through Slick and Slack channels. Manipulation of endogenous PIP_2 levels using nanomolar concentrations of wortmannin, which is known to block PI3 kinase leading to PIP_2 build-up in the membrane, resulted in increased currents through Slick and Slack channels. In contrast, application of micromolar concentrations of wortmannin, which block both PI3 and PI4 kinases leading to PIP_2 depletion from the membrane, resulted in decreased activity of both channels. Treatment with neomycin, a polycation which acts as PIP_2 scavenger and prevents it from interacting with the channels, produced a stepwise reduction in the activity of Slick and Slack channels induced by exogenous PIP_2. In conclusion, the present study demonstrates for the first time that Slick and Slack K^+ channels are regulated by the availability of PIP_2 in the membrane. Therefore, phosphatidylinositol 4,5-biphosphate acts as common activator of the Slo K^+ channel family despite their different structures and physiological roles.

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Coupling of P2Y Mediated Store Depletion and Ca2+ Entry (SOCE) to KCa3.1 (KCnn4/SK4/Ik) Channel Activation in Microglia

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We recently published that SK3/KCa2.3 and SK4/KCa3.1 channels contribute to microglial activation and neurotoxicity via regulation of p38 MAPK activation; and were intrigued that two SK channels are involved in the same outcomes. Cloned SK channels are activated by similar rises in Ca2+; thus, we asked whether they are functionally coupled to different Ca2+ sources. Damaged neurons release ATP, a purinergic receptor ligand that activates microglia by raising intracellular Ca2+. Purinergic responses are mediated by ionotropic P2X or metabotropic P2Y receptors. Here, we stimulated MLS-9 cells (a microglia cell line that expresses both SK3 and SK4) with UTP to trigger P2Y-mediated responses; i.e., depletion of intracellular Ca2+ stores, and subsequent store operated Ca2+ entry (SOCE). In Fura-2 loaded cells, UTP evoked a rapid Ca2+ transient, followed by a second, more sustained rise due to SOCE. The second phase was decreased by the inhibitor 2-APB, which we recently showed blocks Ca2+ release-activated Ca2+ (CRAC) channels in microglia. In perforated-patch recordings, UTP activated a robust current, which was blocked by the SK4 inhibitor, TRAM-34 (but not by the SK3 blocker, apamin), and was therefore, SK4/KCa3.1. SK4 activation by UTP was also inhibited by 2-APB, suggesting that SOCE is required for, and coupled to, SK4 channel activation. SK4 activity was apparently needed for Ca2+ entry and store re-filling; the SK4 blocker, TRAM-34, accelerated the decay in internal Ca2+ after UTP, and reduced the amplitude of a second Ca2+ release from stores evoked by the SERCA pump inhibitor, thapsigargin. Our data support selective coupling of SK4 channels (but not SK3) to Ca2+ entry through SOCE/CRAC. Our current model is that SK4 activation maintains a hyperpolarized membrane potential that helps drive Ca2+ entry through CRAC, facilitating store refilling.

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Identification and Evaluation of Inhibitors of Orai Channels using a Novel Methodology

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Store operated calcium channels (SOCs) play a central role in regulating calcium concentrations in non-excitable cells and are critical for many physiological processes including activation of the immune system. SOCs in lymphocytes are mediated by the ion channel Orai that is activated by the endoplasmic reticulum protein STIM through direct binding. Pharmacological blockers of Orai and STIM would be useful tools for basic research in addition to providing potential treatments for autoimmune disorders. We have developed a novel methodology for generating SOC blockers based on identification and targeting the functional domains of STIM and Orai. We have identified a peptide blocker of Orai that is a potent and specific inhibitor of the channel and a small molecule blocker that can inhibit the channel at low micromolar concentrations. We have shown that the small molecule blocker can inhibit T cell activation both in vitro and in vivo. These studies provide an important set of tools for studying STIM and Orai and lead compounds that could be developed into clinically useful agents.

3509-Pos Board B370

Mathematical Modeling of T-Cell Electrophysiology

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The immune system represents a complex sophisticated network based on specialized immune cells coordinated by fine tuned signaling pathways. The presence of T-lymphocytes, in particular T-helper-cells, is in this context of great importance, but in some medical issues their immunological implication must be repressed. For example, the appearance of graft rejection is a major problem in transplantation medicine. In order to suppress the causative immunological reaction, the pharmaceuticals cyclosporin A (CsA) and tacrolimus (FK506) are in use as standard drugs. Blocking the relevant signaling cascade in activated T-cells and in this way also modulating the calcium influx passing through the calcium-release activated channel (CRAC) in the plasma membrane of T-lymphocytes, both immunomodulators affect electrophysiological processes.

With regard to the significance of electrophysiology in coherence with immunological interactions and pharmacological issues, a mathematical modeling approach is demanded in order to understand the associated ion dynamics in T-lymphocytes, embedded in a systems immunological context. Building on the results from experimental data out of electrophysiological measurements, the intended T-cell model will include single transmembrane protein characteristics in order to represent ion dynamics pattern with the focus on calcium levels on the origin of the CRAC channel under different circumstances. Technically, the background of the projected in silico investigation of T-cell electrophysiology is based on mathematical modeling of the electrophysiology of the pancreatic beta-cell [1]. Starting the simulation with a resting T-cell, the model will then be extended regarding the electrophysiological changes following T-cell activation. Further, the modeling approach will finally provide a starting point for in silico studies relating to the therapeutic immune modulation of ion-conducting proteins in T-lymphocytes.

[1] Meyer-Hermann, Michael E. The Electrophysiology of the ß-Cell Based on Single Transmembrane Protein Characteristics. Biophysical Journal 93, 2007: 2952-2968

Cytoskeletal Protein Dynamics

3510-Pos Board B371

Structure Formation in Active Actin Networks

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The actin cytoskeleton determines local mechanical and structural properties of a eukaryotic cell. To adapt to their environment, constant self-organized